FEBS Letters 588 (2014) 1136–1143

journal homepage: www.FEBSLetters.org

A proneural gene controls *C. elegans* neuroblast asymmetric division and migration

Zhiwen Zhu [a,1], Jianhong Liu [a,b,1], Peishan Yi [a,b], Dong Tian [c], Yongping Chai [c], Wei Li [d], Guangshuo Ou [c,*]

[a] National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Chaoyang District, Beijing 100101, China
[b] University of Chinese Academy of Sciences, Beijing, China
[c] Tsinghua-Peking Center for Life Sciences, School of Life Sciences, Tsinghua University, Beijing 100084, China
[d] School of Medicine, Tsinghua University, Beijing 100084, China

ARTICLE INFO

Article history:
Received 31 December 2013
Accepted 14 February 2014
Available online 28 February 2014

Edited by Jesus Avila

Keywords:
Proneural Gene
Neuroblast
Asymmetric cell division
Cell migration
C. elegans

ABSTRACT

Proneural genes control the generation of neuroblasts from the neuroepithelium, but their functions in neuroblast asymmetric division and migration remain elusive. Here, we identified *Caenorhabditis elegans* mutants of a proneural transcription factor (TF) *lin-32*, in which Q neuroblasts are produced. We showed that LIN-32 functions in parallel with a storkhead TF, HAM-1, to regulate Q neuroblast asymmetric division, and that Q neuroblast migration is inhibited in *lin-32* alleles. Consistently, *lin-32* is expressed throughout Q neuroblast lineage, suggesting that LIN-32 may promote different target gene expression. Our studies thus uncovered previously unknown functions of a proneural gene in neuroblast development.

1. Introduction

Neurogenesis involves the generation of a wide array of neurons and glia cells. In both invertebrates and vertebrates, the neuroepithelial layer consists of cells that have the potential to produce neurons and glia. Neural progenitors delaminate from the neuroepithelium and undergo limited rounds of cell divisions and then differentiate [1,2]. Genetic studies identified a family of the Basic-HELIX-LOOP-HELIX transcription factors as the proneural genes, which are necessary and sufficient to control epithelia-neuron transition [1,3–5]. Recent studies indicated that proneural genes have multiple functions in neural development, including integration of positional information and neuronal identity specification [1].

After delamination from the *Caenorhabditis elegans* epithelium, the Q neuroblast on the right or left side of the L1 larvae undergoes three asymmetric cell divisions to produce an oxygen sensory neuron (AQR or PQR), a mechanosensory neuron (AVM or PVM) and an interneuron (SDQR or SDQL) as well as two apoptotic cells (Fig. 1A) [6,7]. During the second round of asymmetric division, Q neuroblast anterior and posterior daughters, Q.a and Q.p, use distinct cellular mechanisms to generate two daughter cells of different sizes and fates [6,7]. In Q.a division, the asymmetric myosin-based contractility likely pushes cellular content from the anterior to the posterior, reducing the anterior daughter cell size and increasing the posterior daughter size. In Q.p division, the initial displacement of the mitotic spindle may generate different daughter cells. The myosin asymmetry or spindle displacement is respectively used in asymmetric cell divisions of *Drosophila* neuroblasts or *C. elegans* embryos [8–10].

Genetic screens in *C. elegans* identified regulatory components that function at sequential steps to ensure Q neuroblast lineage progression. LIN-32, a basic helix-loop-helix transcription factor (TF), is important for the generation of Q neuroblasts [5]. UNC-86, a POU domain TF, modifies the latent reiterative Q cell lineages [11]. HAM-1, a storkhead TF, controls the expression of a PAR-1-like kinase PIG-1 to regulate myosin polarization during Q.a asymmetric division [12–14]. EGL-44, a TEAD TF, and EGL-46, a Zinc-finger TF, form a complex to instruct the final cell cycle exit [13,15]. EGL-13, a Sox domain TF establishes the correct cell fate during the neuronal differentiation [13,16]. The cellular targets of these TFs or the substrates of the PIG-1 kinase have yet to be identified.

In this study, we performed forward genetic screens to isolate critical factors for Q cell development. We identified two novel alleles of *lin-32* in which Q neuroblast progenies are generated.

* Corresponding author.
E-mail address: guangshuo.ou@gmail.com (G. Ou).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.febslet.2014.02.036
0014-5793/

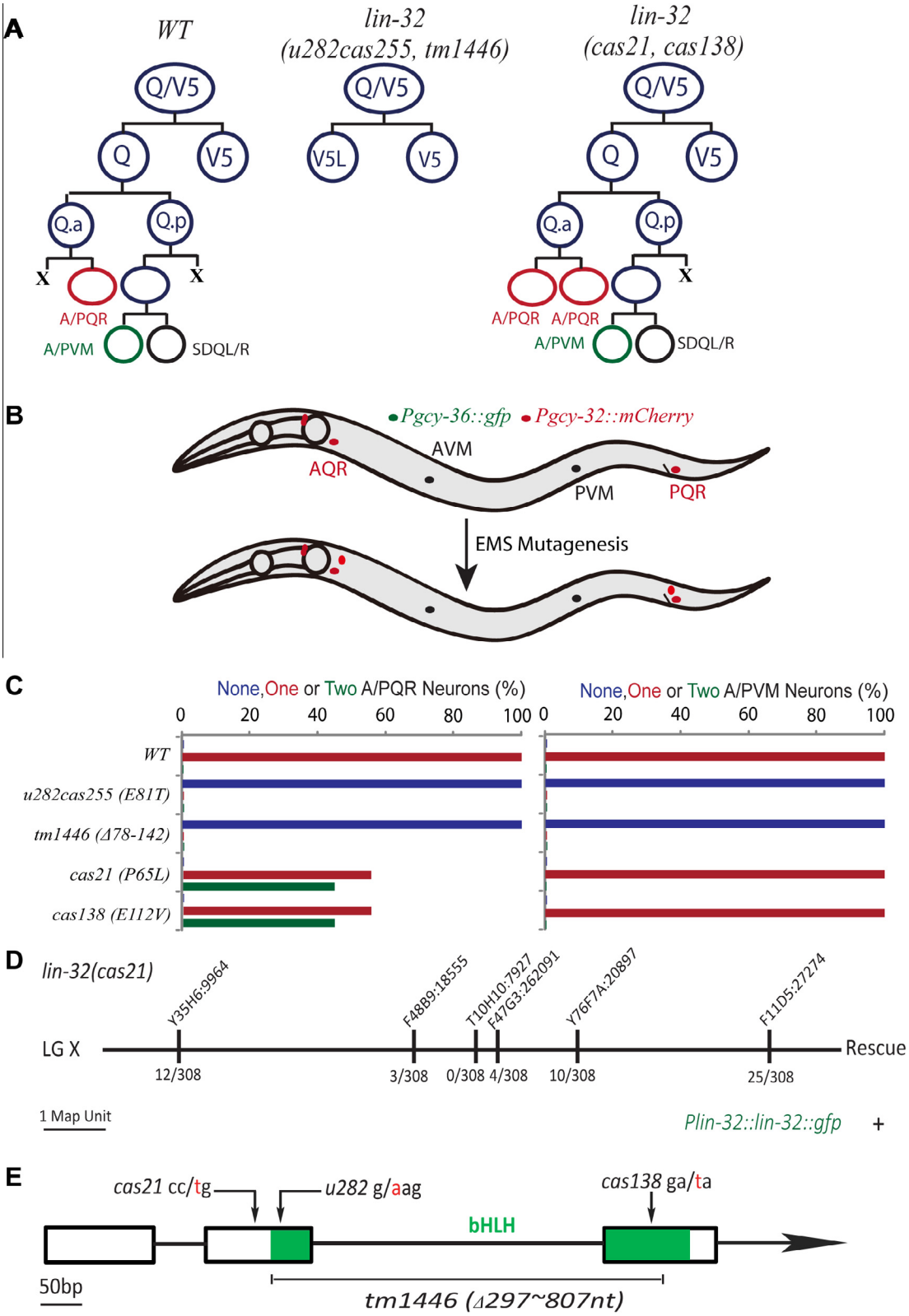

Fig. 1. LIN-32 controls *C. elegans* Q neuroblast development. (A) Q neuroblast lineages in WT and different *lin-32* alleles. In WT animals (left), Q neuroblasts undergo asymmetric divisions to produce three distinct neurons and two apoptotic cells (X). On the left side, QL makes PQR, PVM and SDQL; and on the right, QR produces AQR, AVM and SDQR. (B) Genetic screens isolated *lin-32* mutations with extra neurons from Q.a cell lineages. P*gcy-32::mCherry* labels AQR and PQR (A/PQR, red). The screen details were described in the text. (C) Percentages of none (blue), one (red) or two (green) A/PQR or A/PVM neurons in *lin-32* mutant alleles. $n = 100$. Mutations altered in *lin-32* were indicated. (D) snip-SNP markers (top) and number of recombinants (bottom) during *lin-32 (cas21)* mapping were shown. Rescue of extra A/PQR phenotype in *lin-32(cas21)* mutants by P*lin32::lin-32::gfp*. (E) *lin-32* gene structures. The nucleotide substitutions or deletion in *lin-32* mutant alleles were indicated. bHLH domain was labeled in green. Bar = 50 bp.

Through live cell imaging and genetic analysis, we showed that both alleles are defective in Q cell asymmetric division and migration. Our work provided insights to multiple functions of LIN-32/Atonal in neural development.

2. Results

2.1. Identification of two novel alleles of lin-32 essential for Q neuroblast development

To identify factors that control Q.a cell asymmetric division, we performed genetic screens which were based on a mCherry fluorescence marker. The posterior daughter of Q.a differentiates into an oxygen sensory neuron, AQR or PQR, on the right or left side of the animal (Fig. 1A). We reasoned that the disruption of Q.a asymmetric division can increase the size of Q.aa and allow its survival and differentiation into an oxygen sensory neuron-like cell. Using this strategy, previous genetic screens isolated *ham-1* and *pig-1* in controlling Q.a asymmetric division [12–14]. We used an oxygen sensory neuron specific reporter, P*gcy-32::mCherry*, to screen for mutations with an extra gain of AQR or PQR neuron (Fig. 1B). We identified two mutants, *cas21* and *cas138*, that generate two A/PQR neurons with 46% penetrance (n = 100 for each mutant allele, Fig. 1C). Interestingly, we did not find that an ectopic gain or loss of A/PVM in *cas21* or *cas138* animals, indicating that the Q.a but not Q.p lineage progression is specifically defective in these mutants (Fig. 1C).

We performed single-nucleotide polymorphism mapping, complementation testing, transformation rescue and allele sequencing to clone the gene(s) affected by these mutants (Fig. 1C–E). We showed that *cas21* and *cas138* mutants cause different molecular lesions in the same transcriptional factor, *lin-32*, which is a homolog of vertebrate or *Drosophila* proneural gene atonal (Fig. 1C–E). LIN-32/atonal contains a basic Helix-Loop-Helix domain and was previously shown to control the generation of Q neuroblasts (5). In *lin-32* (*u282*) mutant animals, Q neuroblasts differentiate into an epithelium V5-like cell, which fuses with the epithelial hyp7 cell, resulting in a complete loss of neurons derived from Q cell lineages (Fig. 1A and C) [5]. Our genetic screen uncovered *lin-32(cas255)* which has the mutation identical to that of *u282*. We could not detect progenies derived from Q neuroblasts in the *u282cas255* mutant allele or the *lin-32* deletion allele *tm1446* (Fig. 1C).

2.2. LIN-32 controls Q.a daughter cell size and fate asymmetry

We next applied live cell imaging technique to address which cellular processes are defective in *lin-32* (*cas21* and *cas138*) alleles. Given that *cas21* and *cas138* have identical penetrance in generating extra A/PQR neurons (Fig. 1C), we used *cas21* allele for live imaging analysis. By visualizing the dynamics of GFP-labeled centrosome, mCherry-tagged chromosome and plasma membrane, we showed that Q.a asymmetric division was defective in *lin-32(cas21)* mutants (Fig. 2A–C). Q.a cell normally position its spindle in the cell center, and the small Q.aa and the large Q.ap daughter cells are likely generated by the anterior accumulation of non-muscle myosin II during cytokinesis (Fig. 2C) [6]. In *lin-32(cas21)* mutants, QR.a cells (n = 11) properly position their spindles (Fig. 2A), however, myosin II in the contractile ring is symmetrically distributed (Fig. 2C), generating two equal sized daughter cells (Fig. 2B). For Q.p cell asymmetric division, its spindle is normally shifted to the posterior and myosin II is evenly distributed, and a large Q.pa and a small Q.pp cell are generated in WT or *lin-32(cas21)* mutants (Fig. 2A–C), which is consistent with the normal A/PVM neuron numbers in adult *lin-32(cas21)* animals (Fig. 1C).

To investigate the cell fate of Q.aa in *lin-32(cas21)* mutants, we carried out the fluorescence time-lapse analysis after Q.a division using mCherry labeled Q cell plasma membrane and histone markers. In WT animals, Q.aa is the half size of Q.ap and it undergoes programmed cell death, and we could visualize the degradation process of Q.aa within two hours in both QR and QL lineages (Fig. 3D). However, in *lin-32(cas21)* animals, Q.aa has the similar size to that of Q.ap and it does not undergo apoptosis but survives and grows neurites (Fig. 3D), which is consistent with the extra A/PQR neuron phenotype observed in adult *cas21* animals (Fig. 1C).

2.3. Dynamics of LIN-32::GFP during Q neuroblast development

We studied the dynamics of *lin-32* expression and LIN-32 protein localization during Q neuroblast development. We first constructed a transgenic line expressing a GFP-tagged LIN-32 protein under the control of the *lin-32* promoter (P*lin-32*). We showed that P*lin-32::lin-32::gfp* transgene reduced the extra A/PQR phenotype from 46% (n = 100) to 3% (n = 32) in *lin-32(cas21)* mutant animals, indicating that P*lin-32::lin-32::gfp* is a functional reporter. Our time-lapse imaging analysis demonstrated that LIN-32::GFP was restricted in the nuclei during interphase and that LIN-32::GFP evenly distributed in the cytoplasm of dividing Q.a and Q.p cells (Fig. 3A and C). The dynamic distribution of LIN-32 during asymmetric cell division is consistent with the role of LIN-32 as a transcription factor.

We studied the expression pattern of *lin-32* during Q neuroblast development. Using the same P*lin-32::lin-32::gfp* reporter, we showed that *lin-32* starts its expression in Q neuroblasts and maintains the expression throughout Q neuroblast development. Interestingly, we found that *lin-32* shows distinct expression patterns between Q.a and Q.p lineages. After Q neuroblast division, Q.a and Q.p appear to have the similar level of LIN-32::GFP fluorescence (Fig. 3A and B). However, in the end of Q neuroblast development, Q.a progenies have approximately five or four folds higher expression of LIN-32::GFP than that of Q.p progenies in the right side or left side of the animals (Fig. 3A and B). The dynamic change of *lin-32* expression in Q.a and Q.p lineage is consistent with the essential role of LIN-32 in Q.a but not Q.p asymmetric cell division. As a control, we did not detect any obvious change of the mCherry fluorescence in Q.a or Q.p lineage during Q cell development (Fig. 3A and B).

2.4. LIN-32 functions in parallel with HAM-1/PIG-1 in Q.a lineage progression

We carried out double mutant analysis to address the genetic interactions of LIN-32 and other transcription factors which are essential for Q cell divisions. Our previous studies showed that HAM-1 promotes the expression of the PIG-1 kinase to regulate Q.a asymmetric division and that the EGL-44/EGL-46 transcriptional regulatory complex controls Q.ap cell cycle exit (Fig. 4A) [13]. The *lin-32(cas21), ham-1(gm279)* or *pig-1(gm344)* single mutation causes two-A/PQR neuron phenotype with the penetrance of 46%, 72% or 19% respectively (Fig. 4C). However, in *lin-32(cas21); ham-1(gm279)* or *lin-32(cas21); pig-1(gm344)* double mutants, we showed that the two-A/PQR phenotype is significantly enhanced to 92% or 83% (Fig. 4B and C), suggesting that LIN-32 functions in parallel with the HAM-1/PIG-1 pathway in regulating Q.a lineage progression. We also demonstrated that 16% *lin-32(cas21); egl-44* (*cas6*) or 15% *lin-32(cas21); egl-46* (*cas36*) double mutants produced four A/PQR-like neurons (Fig. 4B and D), indicating that defects of asymmetric cell division in *lin-32* and cell cycle exit in *egl-44* or *egl-46* are additive. Our genetic analysis suggested that LIN-32 functions together with other TFs to additively regulate Q neuroblast development.

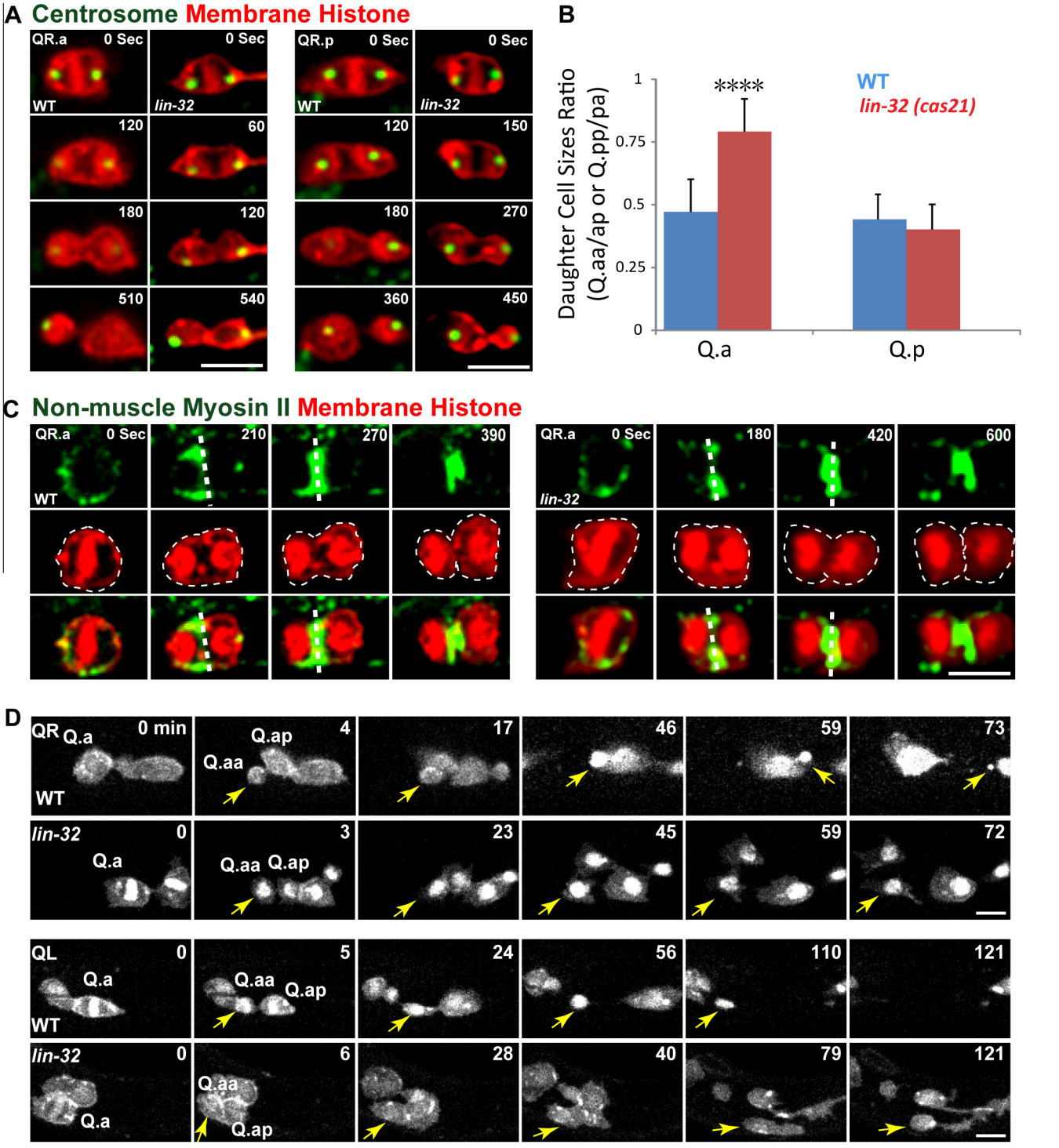

Fig. 2. Q cell asymmetric divisions in *lin-32* mutants. (A) Still images showed QR.a (left panel) and QR.p (right panel) mitotic spindle positioning and daughter cell sizes in the WT (left columns of each panel) and *lin-32 (cas21)* mutant (right columns of each panel) animals. GFP-tagged centrosome protein CMD-1 was used to mark centrosome in green; and plasma membrane and chromosomes in red were labeled by mCherry fused with a myristoylation signal and histone (HIS-24). Time in second. (B) Daughter cell size ratio (Mean ± s.t.d) in WT and *lin-32 (cas21)* mutant. $^{****}P < 0.0001$ by Student's *t*-test, $n = 6–21$. (C) Myosin II (GFP-tagged NMY-2 in green, top panel) distribution in WT (left) and *lin-32 (cas21)* mutants (right). Plasma membrane and chromosomes were labeled in red (mCherry fused with a myristoylation signal and histone, middle panel). Q cell names were adjacent, and cell shapes were outlined. Merged images were at the bottom. Division furrows were indicated with dashed lines in the GFP and merged images. (D) QR.aa (upper panel) and QL.aa (lower panel) underwent apoptosis and were then eliminated in WT (upper rows in each panel), whereas Q.aa cells survived, migrated and then differentiated in *lin-32 (cas21)* mutants (lower rows in each panel). Arrows indicated Q.aa cells in frames. Bar in (A, C and D) = 5 μm. Anterior to the left.

2.5. LIN-32 controls Q cell migration

To examine the function of LIN-32 in Q cell migration, we quantified the final positions of Q cell progenies in adult animals. The integrated *Pmec-4::gfp (zdIs5)*, *Pgcy-32::mCherry (casIs35)* transgenes were used to visualize Q descendants, AVM/PVM and AQR/PQR. Both markers can also illuminate the non-migratory URX and PLM neurons, which serve as land markers to quantify the final position of Q cell progenies (Fig. 5A) [17]. We showed that the anterior migration of QR progenies is normal in *lin-32(cas21)*

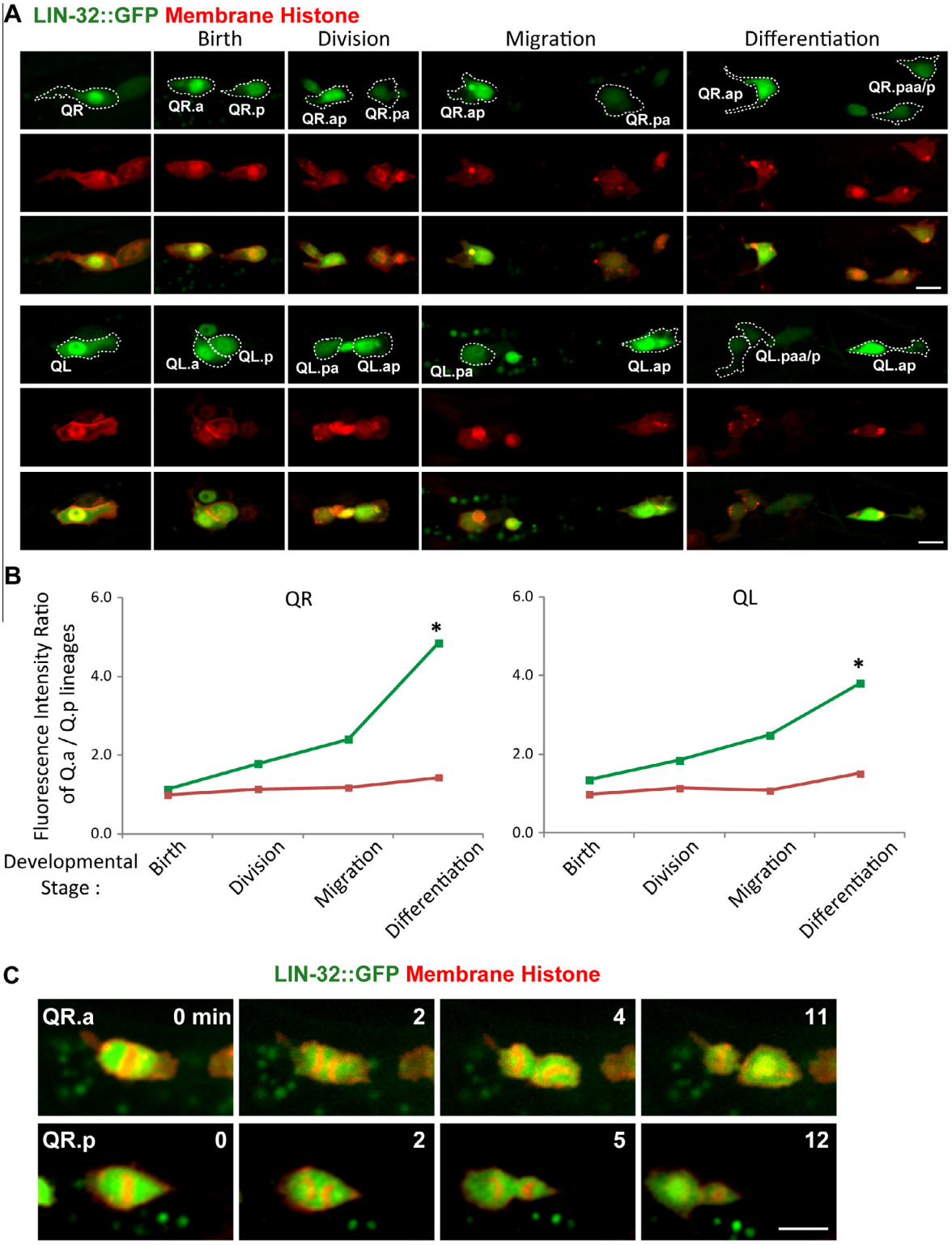

Fig. 3. The expression and subcellular distribution of LIN-32 protein. (A) Still images showed LIN-32 protein expression and subcellular localization in different developmental stages of QR (upper panel) and QL (lower panel) lineages. LIN-32::GFP expression was driven by the endogenous *lin-32* promoter (green, top row, cell shapes were outlined, Q cell names were adjacent). Q cell developmental stages were indicated: Birth for the generation of Q.a and Q.p cells; Division for the stage after Q.a and Q.p division; Migration for Q cell progenies that reach their final destination; Differentiation for Q cell descendants that form neurites. Q cell plasma membrane and chromosomes were marked with mCherry (Red, middle row), and the merged images were at the bottom row of each panel. (B) Quantification of the fluorescence intensity ratio of LIN-32::GFP in Q.a verse Q.p or their progenies (lines in green) at sequential developmental stages as marked in A ($n = 14–35$, each measurement). mCherry fluorescence ratio (lines in red) from the same measurements was used as an internal control. *$P < 0.05$, Student's t test. (C) LIN-32::GFP dynamic distribution in QR.a (upper) and QR.p (lower) asymmetric divisions. Still images represented interphase, metaphase, anaphase, cytokinesis and post-division. Bars in A and C = 5 μm.

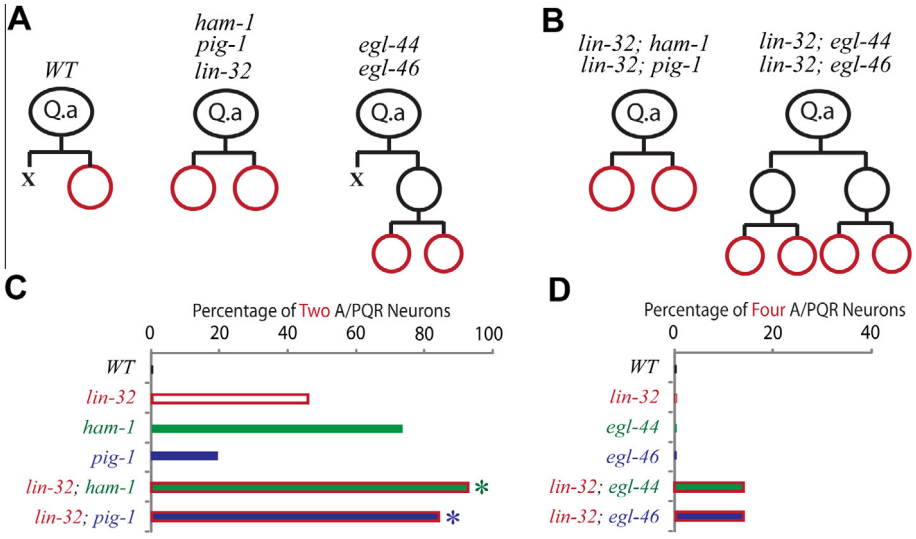

Fig. 4. Genetic interactions of *lin-32, ham-1, pig-1, egl-44, egl-46* in Q cell lineage progression. (A) Summary of Q.a cell phenotype in *lin-32, ham-1, pig-1, egl-44* and *egl-46*. "X": apoptotic Q.aa cells; red circle: A/PQR neurons. (B) Summary of double mutant phenotypes. In *lin-32; ham-1* or *lin-32; pig-1* double mutants, two cells expressed P*gcy-32::mCherry*. In *lin-32; egl-44* or *lin-32; egl-46* double mutants, four cells expressed P*gcy-32::mCherry*. (C and D) Quantifications of the A/PQR phenotype in double mutants. Asters in the left panel indicate that double mutants significantly enhance the single mutant phenotype type. $^*P < 0.05$, χ^2 test (double mutants paired with single mutants). For each data point, $n = 100$ from a single experiment. Four A/PQR neurons were never observed in single mutants.

mutant animals. However, positions of PVM and PQR neurons in the left side of the adult *C. elegans* are significantly shifted towards the anterior in *lin-32(cas21)* mutants and that some PQR neurons even migrate to the position adjacent to AQR (Fig. 5B, the third row, right; Fig. 5C). In contrast, we did not detect any defects of PVM or PQR position in *lin-32(cas138)* mutants, but the anterior migration of AQR and AVM is significantly reduced in these animals (Fig. 5B, bottom, left; Fig 5C). We showed that Q cell migration is normal in *pig-1(gm344)* mutant animals (Fig. 5B, the second row, right; Fig. 5C), suggesting that the defect of asymmetric cell division does not necessarily change the migratory behavior in Q cell lineages. Our data indicated that LIN-32 regulates Q cell migration and that different LIN-32 point mutations distinctly alter the migratory behavior of QL or QR progenies.

3. Discussion

This study identified two novel point mutations of a proneural transcription factor LIN-32 that change the pattern of asymmetric cell division and migration during *C. elegans* Q neuroblast development. Our data indicated that the proneural gene not only plays an essential role in the epithelium-neural progenitor transition but also controls subsequent developmental events during neuroblast lineage progression. Our findings are consistent with the multiple functions of proneural genes in *Drosophila* and vertebrate neurogenesis [1,2].

Different *lin-32* alleles may provide mechanistic insights in how LIN-32 regulates neuroblast development. The deletion allele of *lin-32(tm1446)* is a likely null allele as the deletion largely removes the bHLH DNA binding domain (Fig. 1E). Consistently, Q neuroblast progenies cannot be detected in *tm1446*. Q neuroblast progenies are also eliminated in *lin-32 (u282)* allele and previous studies suggested that the E71T mutation in *u282* allele abolishes the DNA binding activity of LIN-32 [5]. The mutation of *lin-32(cas21)* is located in the N-terminal of LIN-32 and the bHLH domain is not affected, indicating that LIN-32 (P65L) may still bind to DNA. It is unclear how the P65L mutation partially disrupts LIN-32 activity and it is possible that the mutation reduces the interaction of LIN-32 with its cofactors. HLH-2, the *C. elegans* E/daughterless ortholog, was previously shown to be a binding partner of LIN-32 [18], however, we did not find any defects of Q cell development in *hlh-2 (tm1768)* mutants (Ou lab unpublished data), indicating that HLH-2 is the cofactor with LIN-32 not in Q neuroblasts but in other tissues such as *C. elegans* male sensory ray [18]. The E112 V mutation in *lin-32(cas138)* allele occurs in the bHLH domain and the change from E to V may alter the binding of LIN-32 to DNA sequence and reduce the target gene expression.

LIN-32 may promote the expression of three distinct groups of genes essential for the epithelial-neural transition, asymmetric cell division and cell migration. Although the abnormal cell migration might be the consequence of defective segregation of cell fate determinants during Q.a asymmetric division, LIN-32 independently activates the expression of genes involved in asymmetric division and cell migration in Q.p cell lineage since Q.p cell does not show abnormal asymmetric division but its progenies (AVM and PVM) are defective in cell migration in *cas21* or *cas138*. CHIP-seq analysis is necessary to identify the transcriptional regulatory targets of LIN-32 in the future.

Our work demonstrated that mutations in *lin-32*(*cas21* and *cas138*) specifically affect Q.a but not Q.p asymmetric division, providing molecular insights into different division patterns of Q.a and Q.p. The specific function of LIN-32 in Q.a asymmetric division is similar to that of HAM-1. However, distinct from *ham-1* null alleles [13,14], *lin-32* null alleles completely eliminate Q cells. *lin-32* (*cas21* or *cas138*) point mutations used in this study may produce LIN-32 that likely reduces its activity in part. It is possible that other point mutations in LIN-32 can change Q.p division pattern, and we cannot exclude the possibility that LIN-32 is also required for Q.p asymmetric division.

Our study indicated that LIN-32 cooperates with the HAM-1/PIG-1 pathway to regulate the apoptotic daughter cell survival and differentiation. We noticed that Q.a daughter cell sizes are 100% disruptive in *lin-32*, *ham-1* or *pig-1* mutants but only 19–46% of these single mutants generate extra A/PQR neurons, suggesting that the daughter cell size may not fully specify the daughter cell fate. We proposed that LIN-32 and HAM-1/PIG-1 pathway may independently regulate Q.a asymmetric division and the asymmetric segregation of cell fate determinants. The

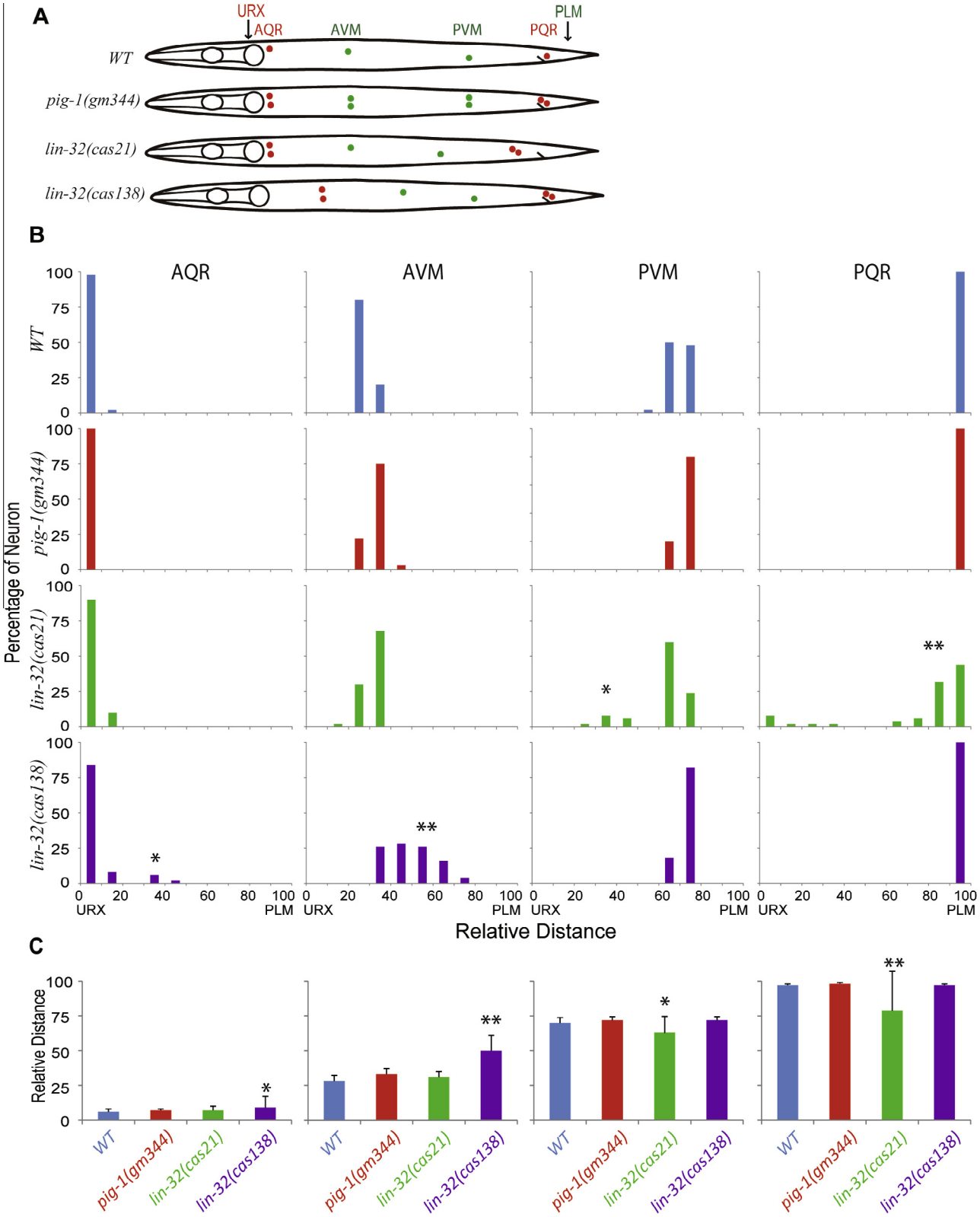

Fig. 5. Q cell migration defects in *lin-32* mutant alleles. (A) Schematics show the final positions of Q cell progenies in WT, *pig-1* and *lin-32* mutants. AVM and PVM (green) or AQR and PQR (red) from Q cell lineages were respectively marked by *zdIs5[Pmec-4::GFP]* or *casIs35[Pgcy-32::mCherry]*. URX (red) or PLM (green) neurons (not shown but pointed with arrows) were used as land markers to quantify the positions of A/PVM and A/PQR. (B) Quantifications of AQR, AVM, PVM and PQR position in WT and mutant backgrounds (indicated on the left). $n = 50$ for each measurement. (C) Statistical analysis of (B). $^{*}P < 0.05$ $^{**}P < 0.01$, by Student's t test.

additive defect of asymmetric segregation of fate determinates in *lin-32; ham-1* or *lin-32; pig-1* double mutants can significantly enhance the survival and differentiation of apoptotic Q.aa cells (Fig. 4B and C).

It is largely unknown about the upstream signaling that activates *lin-32* in Q neuroblast generation. There is growing evidence that the notch signaling pathway plays a vital role in controlling proneural gene in neuronal commitment [1], but we did not

observe any loss of Q neuroblasts in *C. elegans* Notch receptor *lin-12* mutants (Ou lab unpublished results). A recent study showed that the Wnt/beta-catenin pathway is involved in the asymmetric distribution of LIN-32 during the *C. elegans* male ray sublineage [19]. Although the canonic Wnt signaling pathway guides Q cell migration, Q neuroblasts and their descendants are generated properly.

Consistent with various functions of atonal, LIN-32 has multiple roles in neurogenesis. LIN-32 controls the development of *C. elegans* sensory structures such as the male sensory rays, the deirid and postdeirid neurons as well as the CEPD head neurons [5,18,20,21]. The *u282* and *tm1446* alleles of *lin-32* used in previous studies disrupt the first step of neurogenesis, and the contribution of LIN-32 in the subsequent steps of sensory structure development can be examined in the newly isolated *lin-32* alleles. Atonal in *Drosophila* and vertebrates not only promotes the generation of neural progenitors but also integrates position information into neurogenesis and specifies progenitor-cell identity [1]. The exploration of atonal in neural progenitor asymmetric division or migration in *Drosophila* or vertebrates may expand the functional repertoire of this family TF in neurogenesis.

Acknowledgements

We thank *Caenorhabditis* Genetics Center for strains. This work was supported by National Basic Research Program of China to W.L. and G.O. (973 Program, 2013CB945600, 2012CB966800 and 2012CB945002), the National Natural Science Foundation of China to G.O. and W.L. (31222035, 31101002, 31171295 and 31190063), Tsinghua University and the Junior Thousand Talents Program of China to G.O.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2014.02.036.

References

[1] Bertrand, N., Castro, D.S. and Guillemot, F. (2002) Proneural genes and the specification of neural cell types. Nat. Rev. Neurosci. 3, 517–530.
[2] Gotz, M. and Huttner, W.B. (2005) The cell biology of neurogenesis. Nat. Rev. Mol. Cell Biol. 6, 777–788.
[3] Jarman, A.P., Sun, Y., Jan, L.Y. and Jan, Y.N. (1995) Role of the proneural gene, atonal, in formation of Drosophila chordotonal organs and photoreceptors. Development 121, 2019–2030.
[4] Jimenez, F. and Campos-Ortega, J.A. (1990) Defective neuroblast commitment in mutants of the achaete–scute complex and adjacent genes of *D. melanogaster*. Neuron 5, 81–89.
[5] Zhao, C. and Emmons, S.W. (1995) A transcription factor controlling development of peripheral sense organs in *C. elegans*. Nature 373, 74–78.
[6] Ou, G., Stuurman, N., D'Ambrosio, M. and Vale, R.D. (2010) Polarized myosin produces unequal-size daughters during asymmetric cell division. Science 330, 677–680.
[7] Sulston, J.E. and Horvitz, H.R. (1977) Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. Dev. Biol. 56, 110–156.
[8] Cabernard, C., Prehoda, K.E. and Doe, C.Q. (2010) A spindle-independent cleavage furrow positioning pathway. Nature 467, 91–94.
[9] Gonczy, P. (2008) Mechanisms of asymmetric cell division: flies and worms pave the way. Nat. Rev. Mol. Cell Biol. 9, 355–366.
[10] Knoblich, J.A. (2010) Asymmetric cell division: recent developments and their implications for tumour biology. Nat. Rev. Mol. Cell Biol. 11, 849–860.
[11] Chalfie, M., Horvitz, H.R. and Sulston, J.E. (1981) Mutations that lead to reiterations in the cell lineages of *C. elegans*. Cell 24, 59–69.
[12] Cordes, S., Frank, C.A. and Garriga, G. (2006) The *C. elegans* MELK ortholog PIG-1 regulates cell size asymmetry and daughter cell fate in asymmetric neuroblast divisions. Development 133, 2747–2756.
[13] Feng, G., Yi, P., Yang, Y., Chai, Y., Tian, D., Zhu, Z., Liu, J., Zhou, F., Cheng, Z., Wang, X., Li, W. and Ou, G. (2013) Developmental stage-dependent transcriptional regulatory pathways control neuroblast lineage progression. Development 140, 3838–3847.
[14] Frank, C.A., Hawkins, N.C., Guenther, C., Horvitz, H.R. and Garriga, G. (2005) *C. elegans* HAM-1 positions the cleavage plane and regulates apoptosis in asymmetric neuroblast divisions. Dev. Biol. 284, 301–310.
[15] Wu, J., Duggan, A. and Chalfie, M. (2001) Inhibition of touch cell fate by egl-44 and egl-46 in *C. elegans*. Genes Dev. 15, 789–802.
[16] Cinar, H.N., Richards, K.L., Oommen, K.S. and Newman, A.P. (2003) The EGL-13 SOX domain transcription factor affects the uterine pi cell lineages in *Caenorhabditis elegans*. Genetics 165, 1623–1628.
[17] Wang, X., Zhou, F., Lv, S., Yi, P., Zhu, Z., Yang, Y., Feng, G., Li, W. and Ou, G. (2013) Transmembrane protein MIG-13 links the Wnt signaling and Hox genes to the cell polarity in neuronal migration. Proc. Natl. Acad. Sci. U.S.A. 110, 11175–11180.
[18] Portman, D.S. and Emmons, S.W. (2000) The basic helix-loop-helix transcription factors LIN-32 and HLH-2 function together in multiple steps of a *C. elegans* neuronal sublineage. Development 127, 5415–5426.
[19] Miller, R.M. and Portman, D.S. (2011) The Wnt/beta-catenin asymmetry pathway patterns the atonal ortholog lin-32 to diversify cell fate in a *Caenorhabditis elegans* sensory lineage. J. Neurosci. 31, 13281–13291.
[20] Chalfie, M. (1989) *Caenorhabditis elegans* development. Curr. Opin. Cell Biol. 1, 1122–1126.
[21] Doitsidou, M., Flames, N., Lee, A.C., Boyanov, A. and Hobert, O. (2008) Automated screening for mutants affecting dopaminergic-neuron specification in *C. elegans*. Nat. Methods 5, 869–872.